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Erica Ditkoff April 16, 2013

Growth and development in girls and women with classic galactosemia

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a thesis submitted to the Faculty of Emory College of Arts and Sciences
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Abstract

Growth and development in girls and women with classic galactosemia
By Erica Ditkoff

Classic galactosemia results from profoundly impaired function of the enzyme galactose-1-phosphate uridylyltranferase (GALT). Delayed postnatal growth has been reported as a long-term complication of the disorder. The purpose of this investigation was to characterize the growth patterns of girls and women with classic galactosemia through childhood and into adulthood**.** Collected growth information from individuals with classic galactosemia was compared to data from unaffected siblings. The study also aimed to evaluate whether a relationship exists between patient physical growth, predicted residual GALT activity, hormone replacement therapy (HRT) use, and ovarian function reflected in plasma AMH levels.

Results showed that, as a group, girls with galactosemia between the ages of 3 and 14 years had median heights below the 20^{th} percentile. However, as the galactosemic girls grew to become young adults their median heights reached above the 50^{th} percentile. Additionally, galactosemic girls and women between the ages of 3 to 24 years had median BMIs between the 20^{th} and 40^{th} percentiles. However, both affected and unaffected girls and women over the age of 20 years had very similar median BMI percentiles and median height standard deviations.

Girls and women who reported using HRT had slightly higher median height percentiles compared to those who did not undergo HRT. We did not see a relationship between AMH level and either height or BMI among galactosemic girls and young women. Additionally, there did not appear to be a connection between predicted residual GALT activity and either height or BMI among galactosemic girls and young women. However, the number of volunteers for whom we had growth data, AMH levels, and predicted residual GALT activity was very limited.

I hope our findings will lead to a more thorough understanding of growth patterns, and the factors that impact growth of girls with classic galactosemia. Ultimately, this information could assist patients and their health care providers in making informed decisions about potential hormone replacement therapy regimens.

Growth and development in girls and women with classic galactosemia

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Table of Contents

Introduction

Classic galactosemia, a potentially lethal disorder, results from impaired galactose metabolism. Galactose, a monosaccharide, primarily enters a baby's system through diet postnatally, but the sugar is also synthesized endogenously in small quantities. Dairy products, which contain lactose, are the main source of galactose in our diets throughout life. Lactose, a disaccharide, is broken down in the gut into glucose and galactose through a hydrolysis reaction. However, free galactose is also found in some fruits and vegetables such as tomatoes and legumes.

Galactose Metabolism

Galactose is metabolized in all organisms studied, ranging from bacteria to mammals, through the Leloir pathway (Frey 1996). The three enzymes involved in the pathway are galactokinase (GALK), galactose-1-phosphate uridylyltransferase (GALT), and UDP-galactose 4'-epimerase (GALE). GALK phosphorylates galactose to produce galactose-1-phosphate. GALT then transfers UMP from UDP-glucose to galactose-1-phosphate, releasing glucose-1-phosphate and producing UDP-galactose. Finally, GALE interconverts between the metabolites UDP-galactose and UDP-glucose. Impairment of any of the three enzymes involved in the Leloir pathway will result in a form of galactosemia, though the symptoms and severity depend on which enzyme is impaired, and the degree of the impairment (Fridovich-Keil 2006).

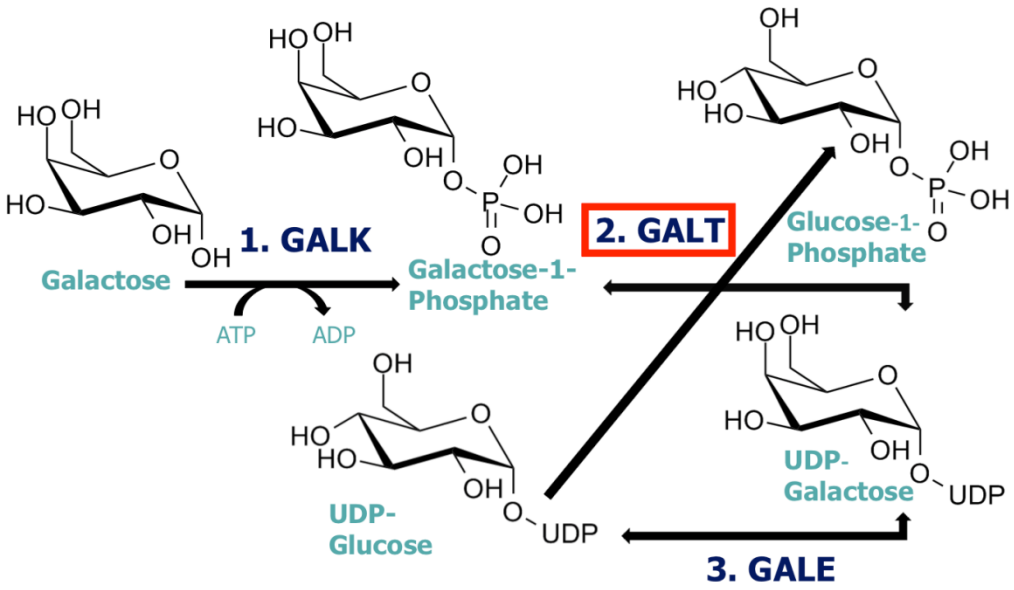

Figure 1: The Leloir Pathway of Galactose Metabolism. Deficiency in the enzyme GALT, outlined in red, results in classic galactosemia. (Structures of galactose and other metabolites were taken from Creative Commons).

The Genetics of Classic Galactosemia

The most prevalent form of life-threatening galactosemia is classic galactosemia. Classic galactosemia affects approximately one in every 47,000 live-born infants (Sanders et al 2009; Suzuki et al 2001). The autosomal recessive condition results from a deficiency in the function of the enzyme GALT. More than 250 different sequence variants have been identified in the *GALT* alleles of patients with ostensibly classic galactosemia (Calderon et al 2007; http://arup.utah.edu/database/GALT/GALT_welcome.php); however, the functional significance of some variants is better understood than others. Most of the known *GALT* mutations are missense point mutations. However, a number of silent, nonsense, and noncoding variants have also been identified. Additionally, various polymorphisms, a large deletion of approximately 5kb, and other smaller deletions, have also been reported. Novel alleles continue to be identified and reported (Fridovich-Keil and Walter 2008). While some individuals with classic galactosemia are homozygotes for a single allele, most are compound heterozygotes having two

different mutant alleles (Fridovich-Keil and Walter 2008). Although most *GALT* mutations are quite rare, a few are seen at a relatively high frequency, at least in some populations. It is these common alleles, such as Q188R in European populations and S135L in African populations, that are likely found in homozygotes.

Understanding the Functional Significance of GALT Variants

There have been a variety of approaches taken to better understand the functional implication of the different *GALT* variants. Six strategies that have been applied in galactosemia research include: (1) patient whole-body studies of galactose oxidation (Barbouth et al 2007; Berry et al 2000; Berry et al 1997), (2) metabolic or enzymatic studies of cultured patient samples or cells (Fridovich-Keil et al 1993), (3) studies of nonhuman mammalian cells engineered to express human *GALT* alleles in addition to their endogenous GALT (Ashino et al 1995), (4) studies of yeast cells engineered to express human GALT in place to their endogenous GALT (Riehman et al 2001), (5) studies of recombinant hGALT proteins isolated from yeast or *E. coli* (Crews et al 2000), and (6) *in silico* predictions derived from the known characteristics of amino acids and knowledge of sequence or structural homology. Through these various methods, a number of identified *GALT* alleles have been characterized and it is now evident that while some alleles are true nulls, many are hypomorphs with at least the potential for residual activity (Fridovich-Keil and Walter 2008).

None of the above strategies is flawless, and at times results of different approaches have been contradictory. For example, COS (CV-1 in Origin expressing SV40 genetic material) cell studies showed that the Q188R GALT allele retains approximately 10 percent of wild-type activity (Reichardt et al). Interestingly, enzyme assays from patient samples and cells (Fridovich-Keil et al 1993), studies of galactose oxidation in patients (Berry et al 2000), and studies using a

null-background yeast expression system (Riehman et al 2001; Fridovich-Keil et al 1993) all demonstrated a complete absence of residual GALT activity associated with the Q188R allele (Fridovich-Keil and Walter 2008). However, each strategy has strengths as well as weaknesses, and when used in pairs or other combinations they have served as useful tools in acquiring information regarding the functional significance of patient *GALT* alleles.

The investigation described in this thesis made use of the null-background yeast expression system to determine predicted residual GALT activity based on volunteers' genotypes. The results from yeast studies often match up well to the results from corresponding patient samples or cells (Fridovich-Keil and Walter 2008). The yeast expression system allows for studies of patient alleles of *GALT* that have resulted from missense or nonsense coding mutations. In the yeast system, patient alleles can either be studied individually or in combination with other alleles (Fridovich-Keil and Walter 2008). Yeast studies of GALT enzymes expressed from single patient alleles have shown a spectrum of activities ranging from essentially null to nearly 10 percent of wild-type function (Riehman et al 2001). It has been shown that the severity of certain long-term outcomes might be influenced by residual GALT activity (Ryan et al 2013).

Acute and Long-Term Effects

Once a newborn with classic galactosemia is exposed to a milk-based diet, he or she will begin to exhibit the acute symptoms of classic galactosemia which include jaundice, vomiting, diarrhea, cataracts, hepatosplenomegaly, and *Escherichia coli* sepsis (Fridovich-Keil and Walter 2008). Without dietary intervention, these early symptoms could ultimately lead to the newborn's death. However, if put on a strict and long-term diet of restricted galactose intake, an

individual with classic galactosemia is relieved of the early symptoms and can avoid the lethality of the disease.

Although a lactose-free diet allows an individual to survive the disorder, long-term complications often occur. The most common long-term problems associated with classic galactosemia are speech and cognitive disabilities (Fridovich-Keil 2006; Waggoner et al 1990). Additionally, primary ovarian insufficiency is seen in >80% of girls and women (Sanders et al 2009; Fridovich-Keil 2006; Waggoner et al 1990). Other complications that have been observed include ataxia, decreased bone density, and delayed growth (Fridovich-Keil 2006; Waggoner et al 1990).

Previous Growth Studies

Waggoner and colleagues previously gathered information about 350 individuals with galactosemia to investigate various long-term outcomes of the disorder, including growth. That study found that in many subjects, childhood and early adolescent growth were severely delayed. However, results of the study also indicated individuals with galactosemia often continued growing through the late teens so that ultimately their final heights were normal (Waggoner et al 1990). Additionally, the study found that mean-heights-for-age percentiles were significantly lower for females than for males at the age ranges of 5 to 6 years, 7 to 9 years, and 10 to 12 years (Waggoner et al 1990). Overall, the results of the investigation indicated that a growth delay is present in both males and females with galactosemia, although the delay appears more prominent in females.

Panis and colleagues also examined growth in individuals with galactosemia, specifically in 40 Dutch children with classic galactosemia. Although normal prenatal growth was observed, postnatal growth appeared to be affected (Panis et al 2007). In the limited population of subjects,

most patients' predicted final heights were less than their target heights, although target heights could be reached for the subjects who grew past the age of 18 years (Panis et al 2007). The results of the investigation also indicated that females had a decreased growth velocity (Panis et al 2007).

Waggoner et al hypothesized that the growth delay in girls might be a consequence of the hormonal background that is associated with ovarian insufficiency. Similarly, Panis et al suspected that suboptimal hormonal replacement in girls could lead to the observed decreased growth velocity. Often, girls with galactosemia require estrogen and/or progesterone replacement therapy to finish puberty and achieve normal secondary sexual characteristics and regular menstrual cycles (Sanders et al 2009; Rubio-Gozalbo et al 2006). However, elevated estrogen exposure from hormone replacement therapy could lead to inadvertently impaired growth due to consequential premature growth plate senescence (Weise et al 2004). In addition to suboptimal hormonal replacement therapy, Panis et al also hypothesized that the decreased IGF-I and IGFBP-3 levels observed in girls could lead to decreased growth velocity (Panis et al 2007).

More recently, Batey and colleagues have also expressed suspicion that inadequate hormone replacement therapy for primary ovarian insufficiency could result in other growth-related complications such as low bone mineral density (BMD). Specifically, Batey et al observed an inverse relationship between gonadotropin levels and spinal BMD in women with classic galactosemia (Batey et al 2012). It is possible that lower dose hormone replacement therapy, which would cause higher gonadotropin levels, may put women at higher risk for low BMD. As a result, it is becoming more evident that the hormone replacement therapy given to girls with galactosemia experiencing primary ovarian insufficiency might have implications

beyond pubertal development and should be extensively studied in order to determine an ideal treatment regimen.

My project

The goal of my investigation was to expand upon the previous studies of growth patterns in girls and woman with classic galactosemia, testing whether height or BMI would show any relationship with an established marker of ovarian function, Anti-Müllerian hormone (AMH), and also testing whether predicted residual GALT activity or hormone replacement therapy use would show any impact on growth in galactosemic girls. To address these questions we collected growth information from girls and women with galactosemia and their family members through a combination of direct measurement of heights and weights, collecting growth and puberty survey responses, and requesting that patients send us their pediatric growth charts. The collected data were divided by age range and growth information from individuals with galactosemia were compared with corresponding data from unaffected siblings and the general population. The data were examined to determine whether relationships exist among female patient growth outcome, predicted residual GALT activity of the GALT alleles present, hormone replacement therapy use, and AMH levels.

Whether a relationship exists between growth in girls and their AMH level is an intriguing question to address because AMH can be used to measure ovarian function (Sanders et al 2009). Additionally, AMH is valuable when examining individuals with galactosemia, because the hormone's levels are essentially unaffected by therapies such as oral contraceptives and gonadotropin treatments (Sanders et al 2009). Furthermore, AMH levels are relatively constant through a woman's menstrual cycles (Sanders et al 2009).

The goal of the investigation is to lead to a more thorough characterization and understanding of growth patterns of girls and women with classic galactosemia. Additionally, through the information from girls and women about their history of hormone replacement therapy and ovarian function, it may be possible to obtain a better understanding of the biochemical factors that contribute to growth in girls with galactosemia. Ultimately, this information and studies that follow could serve to assist patients and their families and health care providers in making better informed decisions about hormone replacement therapy options.

Methods

Volunteer Recruitment

Sixty-four girls and women between the ages of 3 - 58 years old and thirty-five unaffected sisters of these volunteers between the ages of 3 - 56 years old participated in the investigation. Subjects were excluded from the study if they faced health issues besides galactosemia that could potentially exacerbate poor growth (e.g. if an individual was on corticosteroids). Some study volunteers elected to partake in the study by self-referral through a family support group (the Galactosemia Foundation); other volunteers were recruited by referral from a health care provider. Additionally, some subjects who provided growth data were asked to participate in the study because they had been diagnosed with classic galactosemia through newborn screening. Participants in the study with classic galactosemia had previously been clinically diagnosed with the disorder either through a red blood cell GALT assay or through GALT genotyping, or both. The study was reviewed and approved as part of Protocol eIRB00024933 (PI: JL Fridovich-Keil) by the Emory University Institutional Review Board. Informed consent, and assent when appropriate, was obtained from each study volunteer or their

parent/guardian. Additionally, if appropriate, authorization was obtained to contact healthcare providers seeking relevant medical information.

Gathering Growth and Puberty Data

Volunteers were asked to fill out a growth survey and if possible submit pediatric growth charts. The growth survey inquired about various growth-related topics including current heights and weights, family members' heights and weights, vitamin supplement intake, and bone health. Although most subjects submitted a growth survey, only 13 submitted actual growth charts. Additional growth information was collected at the Bi-Annual Galactosemia Foundation Conference, where heights and weights of 31 female study volunteers with classic galactosemia and of a number of parents and unaffected siblings were measured. Female volunteers 8 years or older were also asked to fill out a puberty survey. The puberty surveys were used to assess subjects' pubertal development and to gather information about whether subjects were receiving, or had received hormone replacement therapy.

Calculating Height Percentiles and Standard Deviations, and BMI Percentiles

Height percentiles and standard deviations from the general population median were calculated using the program GenenCalc. BMI percentiles were calculated using the CDC's BMI Percentile Calculator for Child and Teen (http://apps.nccd.cdc.gov/dnapabmi/). For volunteers over the age of 20, adult percentiles were determined by assigning them the age of 19 years and 11 months, because typically percentiles are only calculated for individuals between the ages of 2 and 20 years.

GALT Genotyping and Residual Activity

As previously described in a scholastic study conducted by Ryan et al, some subjects' *GALT* genotypes were obtained through the collection of medical history information which

included *GALT* genotype analyses. For the study volunteers whose *GALT* genotypes were unknown, saliva or bloods samples were used to isolate DNA and determine *GALT* genotype through DNA sequence analysis, as will be described elsewhere (Gleason et al, unpublished).

All *GALT* mutations that impact the coding region were evaluated for residual activity using the previously described null-background yeast expression system for the human GALT enzyme (Fridovich-Keil and Jinks-Robertson 1993; Chhay et al 2008). Volunteers were excluded from analysis if they carried one or more alleles with non-coding mutations only. Ultimately, a "predicted residual GALT activity" value was determined for each volunteer through averaging the predicted activity levels of the individual's two *GALT* alleles. Of the volunteers in the study, 23 girls and women had known *GALT* genotypes that could be modeled in the yeast, and thus assessed for predicted residual GALT activity.

AMH Measurements

AMH levels in volunteers' plasma or serum samples were determined as previously described by Sanders et al (Sanders et al 2009). The AMH/MIS ELISA kit from Diagnostic System Laboratories (Webster, TX) was used for these assays. Assays were executed by the Reproductive Endocrine Unit Laboratory, Massachusetts General Hospital (Boston) or by the Biomarkers Core Facility of Yerkes Primate Research Center at Emory University (Atlanta, GA). Duplicate samples sent to both laboratories yielded indistinguishable results (Sanders et al 2009).

Analysis

Data were compiled into excel spreadsheets and then exported to and graphed in the program SAS JMP 10. Due to the low number of subjects involved in most comparisons, no meaningful statistical analyses were performed.

Results

Growth results

Data were collected from sixty-four girls and women with classic galactosemia and from thirty-five unaffected sisters of these volunteers. Through the submission of pediatric growth charts, thirteen volunteers provided longitudinal information about height and weight so BMI percentiles for these volunteers at different ages could be calculated. As a result, a total of 127 height and weight values from volunteers with classic galactosemia were used to calculate height percentiles, height standard deviations, and BMI percentiles.

Height and BMI as a function of age

Figure 2 shows height percentiles of volunteers with classic galactosemia as a function of their age. Similarly, Figure 3 shows BMI percentiles of volunteers with classic galactosemia as a function of their age. Figure 2 illustrates that girls with galactosemia between the ages of 3-14 years had median heights below the 20^{th} percentile for the general population, but that as they grew older their median heights reached above the 50^{th} percentile. Figure 3 shows that galactosemic girls and women between the ages of 3-24 years had median BMIs between the 20^{th} and 40^{th} percentiles.

Height percentile as a function of age
(N for these groups= 15 (33 points), 13 (29 points), 22 (34 points), 12 (13 points), 4 (4 points), and 11 (11 points))

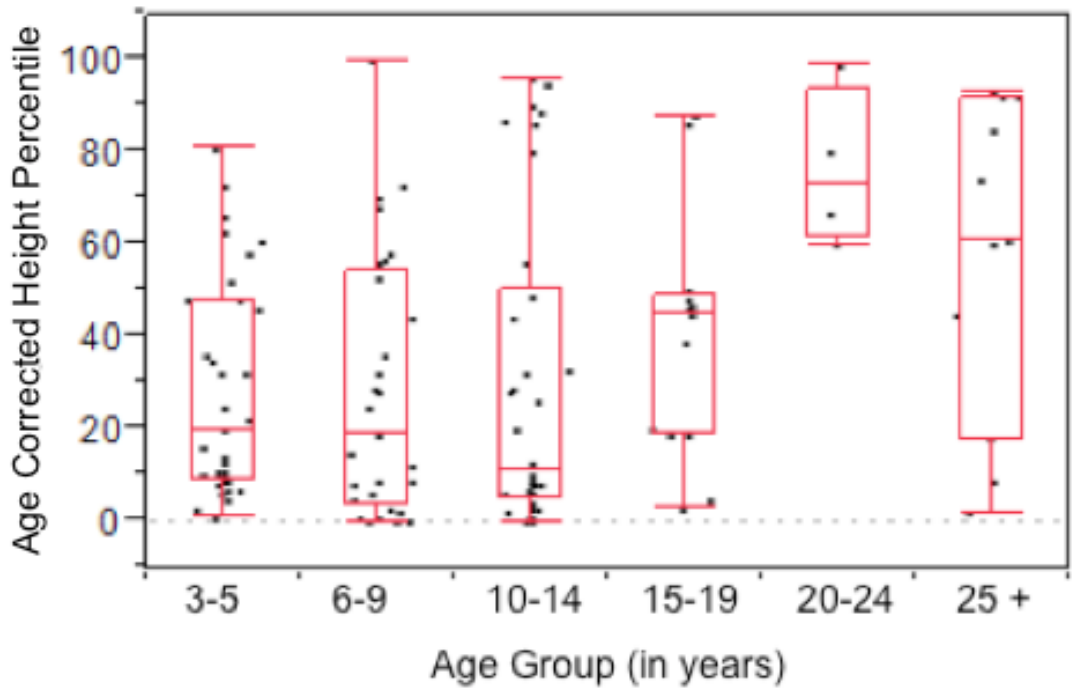

Figure 2. For each of the six data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Girls with galactosemia between the ages of 3 and 14 years had median heights below the 20th percentile, but that as they grew older their median heights reached above the 50th percentile. *Note: for some of the data sets, the 95% limits nearly coincided with the 75th and/or 25th percentile limits, so that only very small whiskers are seen.*

BMI percentile as a function of age group
(N for these groups= 15 (35 points), 13 (30 points), 22 (34 points), 12 (13 points), 4 (4 points), and 11 (11 points))

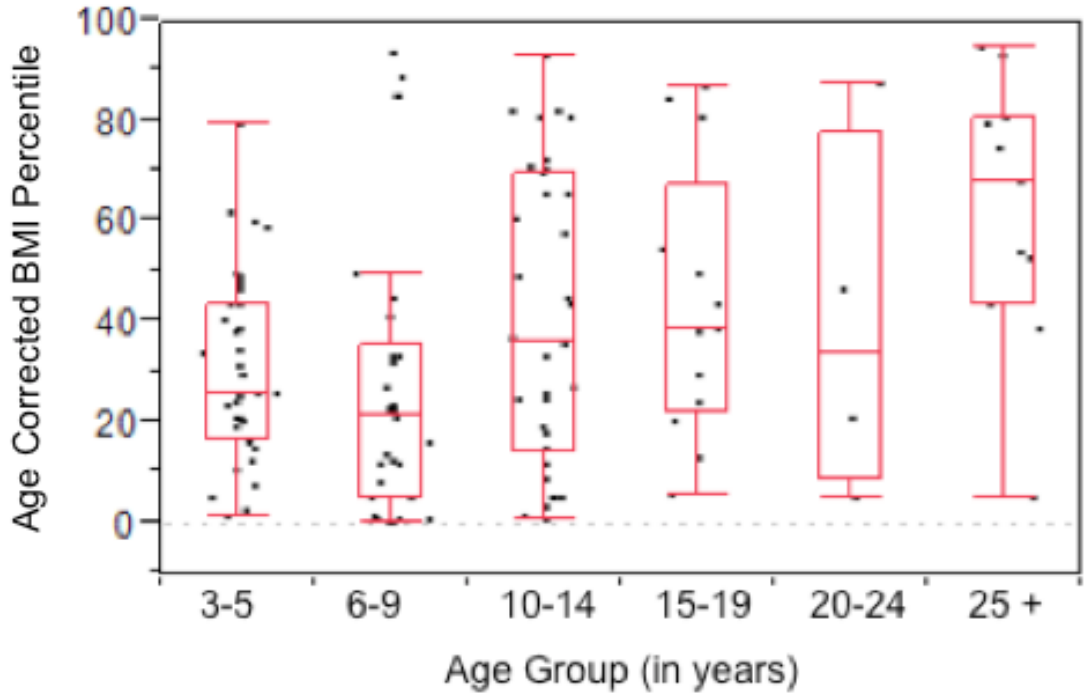

Figure 3. For each of the six data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Females between the ages of 3 and 24 years had median BMIs between the 20th and 40th percentiles.

Height and BMI as a function of residual GALT activity

Twenty girls and women with classic galactosemia in this study had known *GALT* genotypes, and thus were each assigned a value of predicted residual GALT activity. Based on their predicted residual GALT activities, volunteers were stratified into two groups: one group including individuals with <0.4% of wild-type predicted residual GALT activity, and the other group included individuals with ≥0.4% of wild-type predicted residual GALT activity. The cut-off was set at this point because it allowed for the greatest number of volunteers to fit into the higher predicted activity group without too closely approaching the enzymatic assay's threshold

of detection. The numbers of volunteers who fit into the <0.4% predicted residual GALT activity and ≥0.4% category, respectively, were fourteen and six. To prevent conclusions from being skewed by a single volunteer with more data points available than others, only one height and weight measurement was used from each volunteer with a predicted residual GALT activity value. Figures 4 and 5, respectively, show height percentile and BMI percentile as a function of residual GALT activity. Predicted residual GALT activity did not appear to influence height or BMI of these volunteers, although the number of volunteers for whom we had growth data and predicted residual GALT activity values was limited and so no meaningful statistical tests were possible.

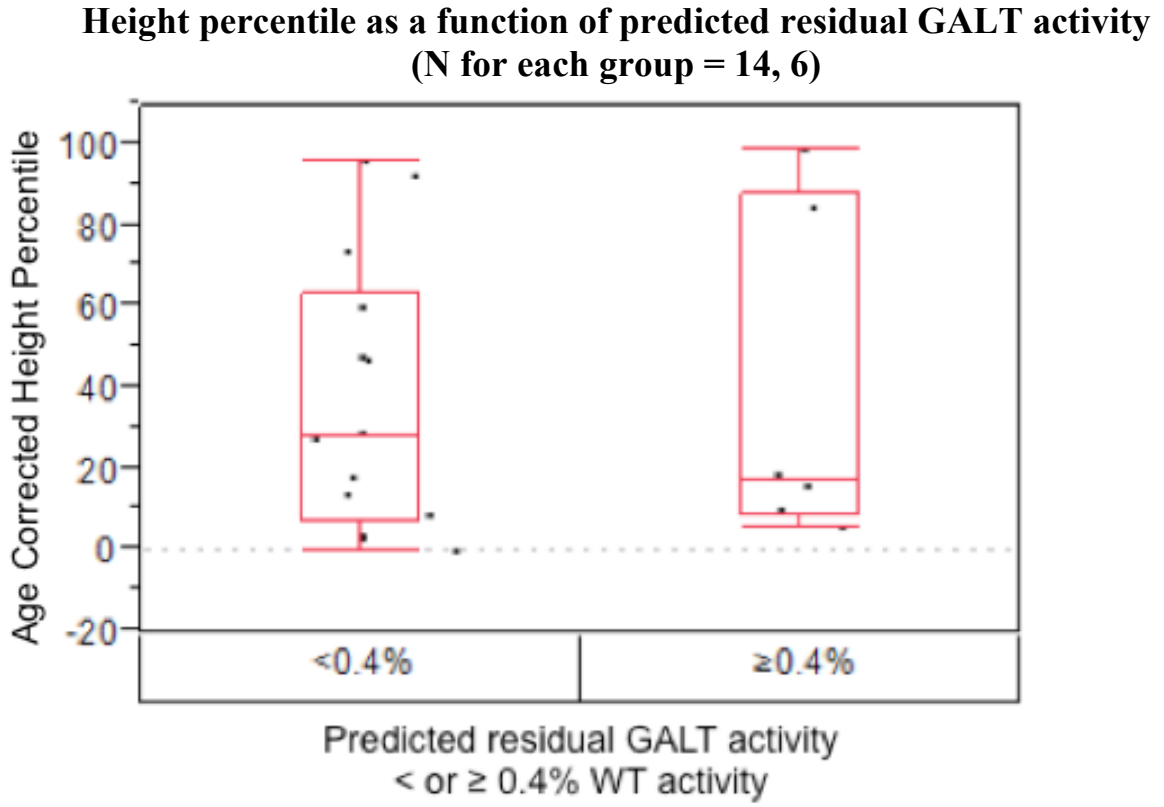

Figure 4. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Predicted residual GALT activity did not appear to influence female height, although the number of volunteers for whom we had growth data and predicted residual GALT activity values was limited.

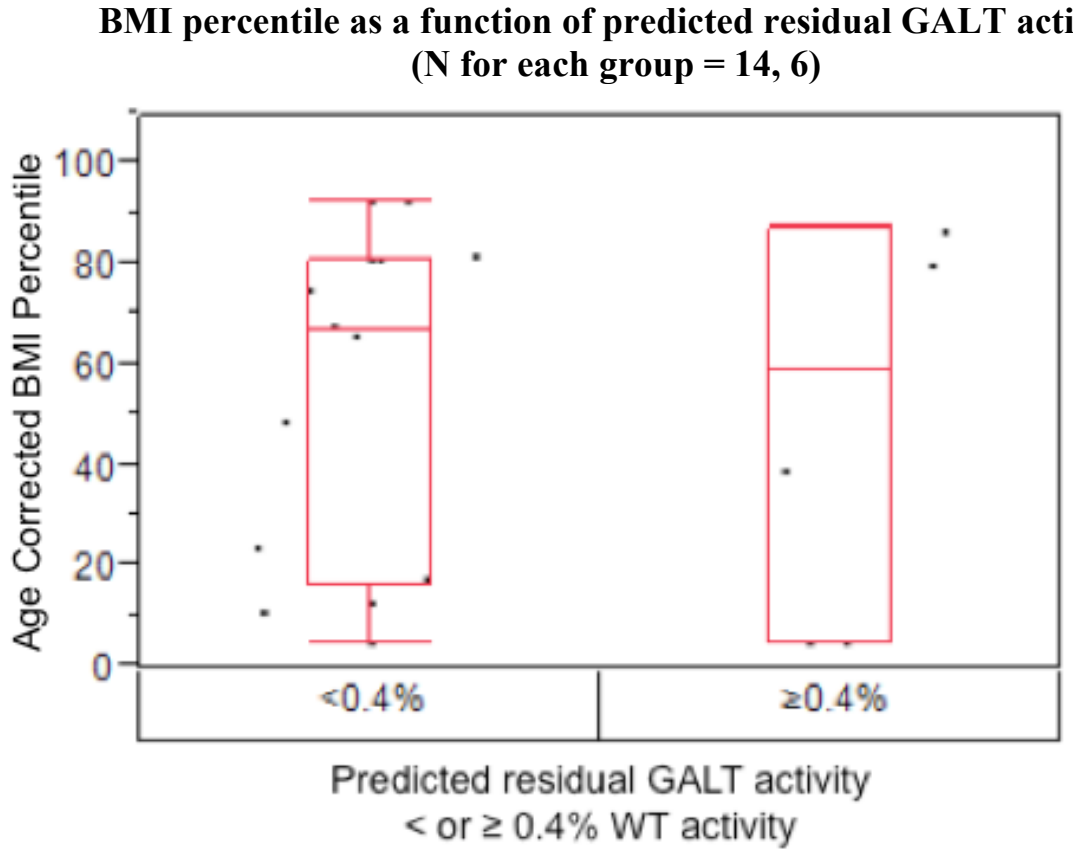

Figure 5. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Predicted residual GALT activity did not appear to influence female BMI, although the number of volunteers for whom we had growth data and predicted residual GALT activity values was limited. *Note: for the ≥.4% data set, a 95% limit is coincided with the 25th and 75th percentile limits, so that little or no whiskers are seen.*

Height and BMI as a function of AMH levels

Twenty-one girls and women with classic galactosemia in this study previously had their AMH levels determined. Based on these AMH levels, volunteers were again stratified into two groups. One group included individuals with AMH levels <0.1μg/L, and the second group included individuals with AMH levels ≥0.1μg/L. Similarly to the predicted residual GALT activity stratification, the cut-off was set here to maximize the number of volunteers in the second group without too closely approaching the assay's limit of detection. Figures 6 and 7, respectively, show height percentile and BMI percentile as a function of AMH level. Because

only six out of twenty-one volunteers with determined AMH levels were in the second category, each individual with a determined AMH level only was matched to a single height percentile or BMI percentile. AMH level did not appear to influence height or BMI of these volunteers, although the number of volunteers for whom we had growth data and AMH level was limited and so no meaningful statistical tests were possible.

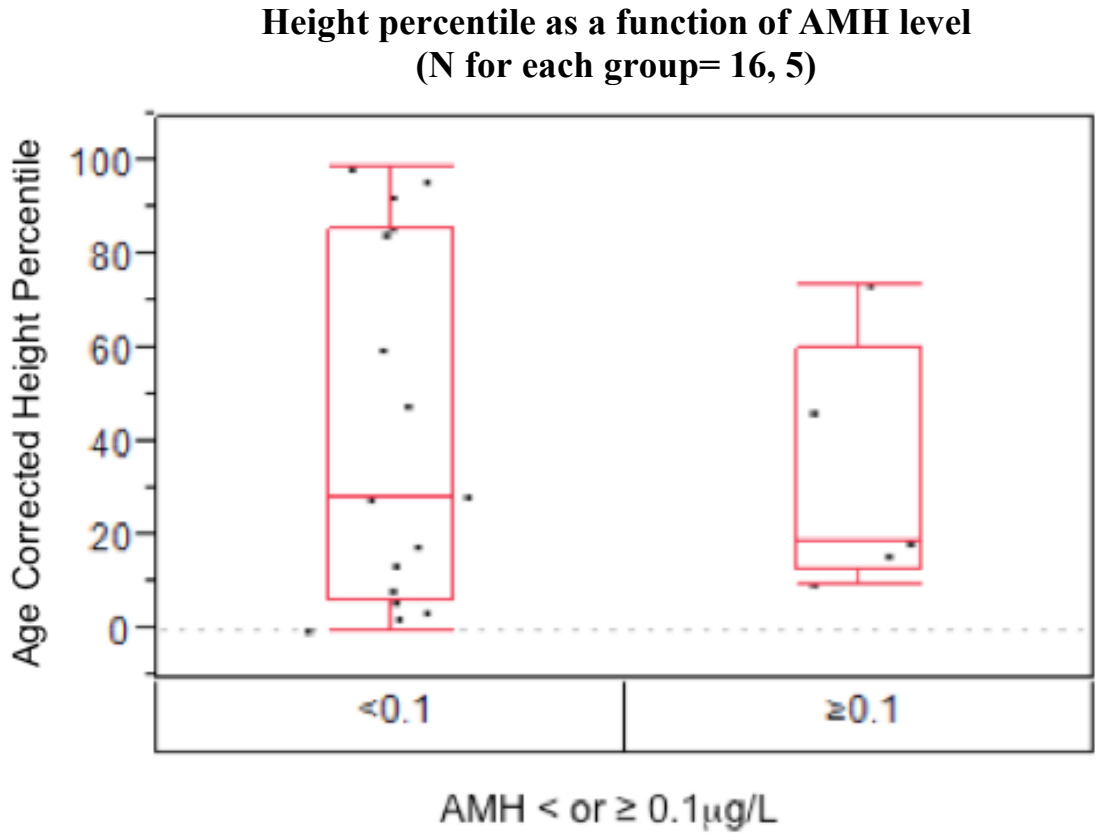

Figure 6. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). AMH levels did not appear to influence female height, although the number of volunteers for whom we had growth data and AMH levels was limited.

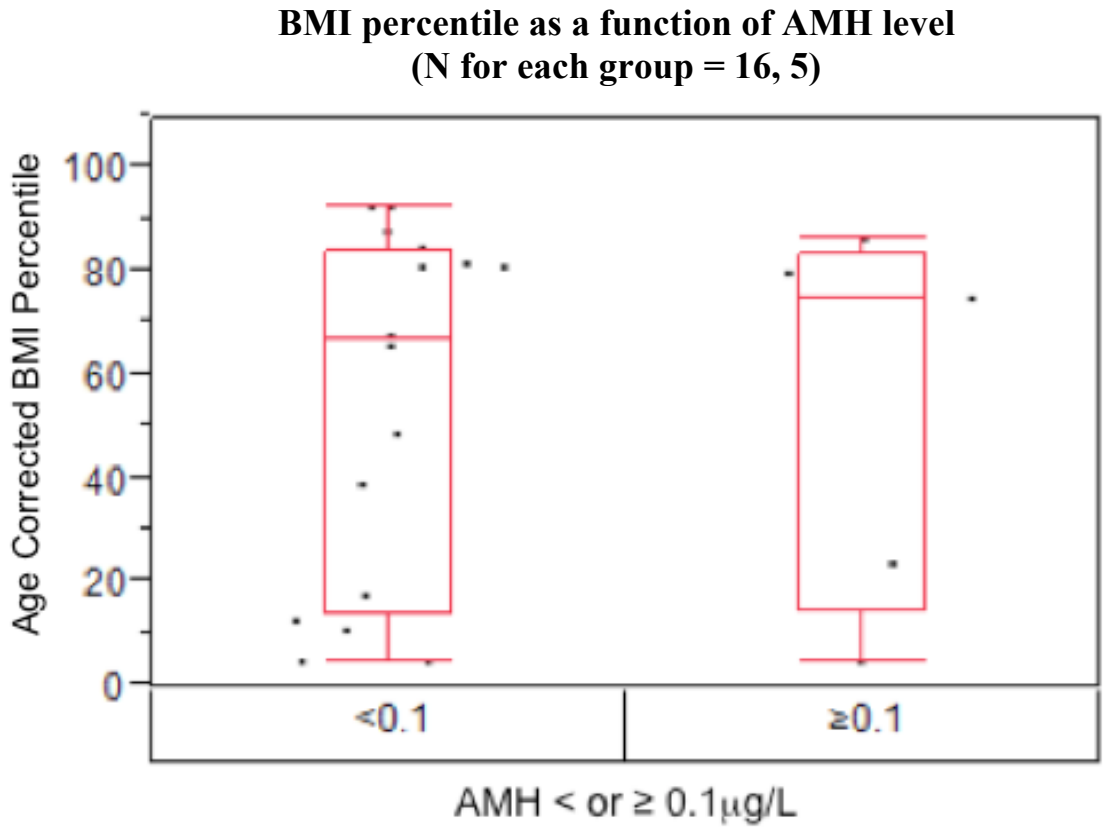

Figure 7. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). AMH levels did not appear to influence female BMI, although the number of volunteers for whom we had growth data and AMH levels was limited.

Heights and BMIs in patients and controls over 20

Twenty out of the thirty-five control siblings were over the age of 20 years. As a result, I decided to compare the heights and BMIs of these controls with the fifteen women with galactosemia who also fell into this age range. Figures 8 and 9, respectively, show height standard deviations from the general population median and BMI percentiles in both women with classic galactosemia and control adult siblings over the age of 20 years. Galactosemic women and their unaffected sisters over the age of 20 years had very similar median height standard deviations and median BMI percentiles.

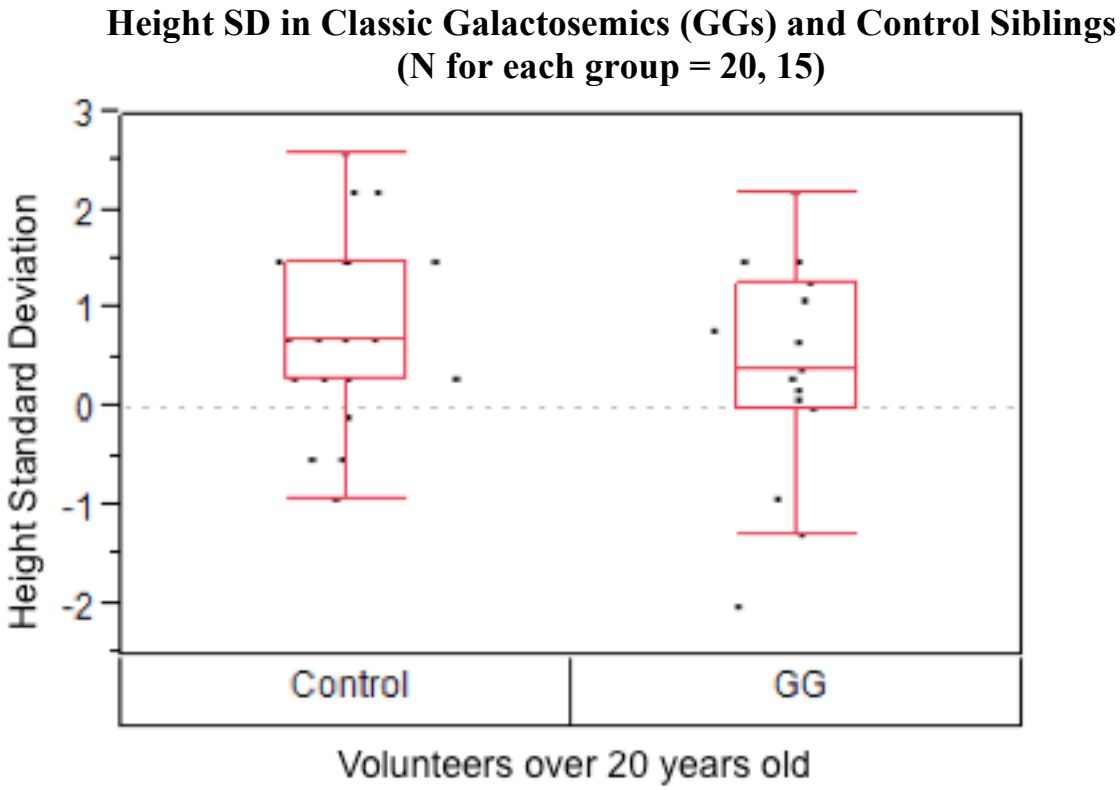

Figure 8. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Females and control siblings over the age of 20 years had very similar median height standard deviations from the general population median. *Note: GG represents individuals with classic galactosemia.*

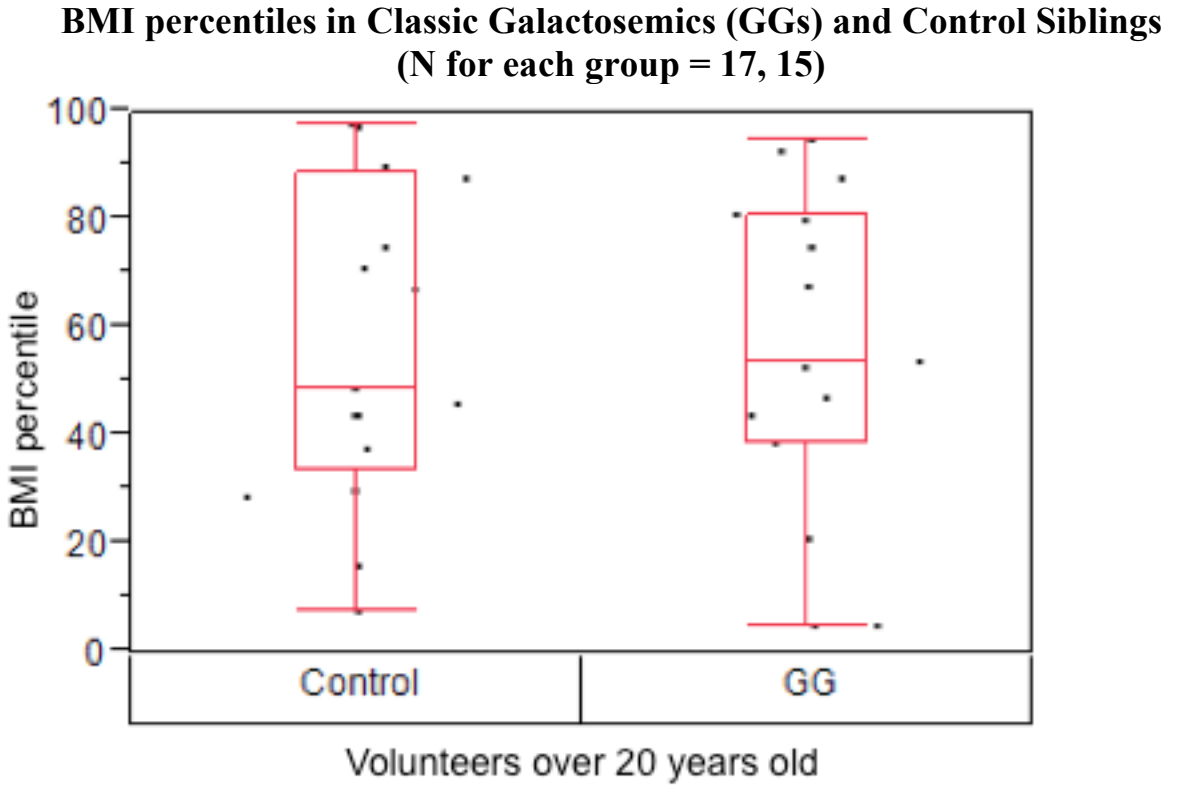

Figure 9. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Affected women and control siblings over the age of 20 years had very similar median BMI percentiles. *Note: GG represents individuals with classic galactosemia.*

Results from puberty surveys

Forty-one girls and women with classic galactosemia between the ages of 8 and 58 years (average age 22.7 years) completed puberty surveys. Information from the puberty survey regarding volunteers' hormone replacement therapy use is summarized in Table 1. Over half of the volunteers (53.6%) who filled out the puberty survey were or are currently on hormone replacement therapy. Hormone replacement therapy was initiated between the ages of 10 and 26 years for these volunteers, with an average age of initiation of 16.4 years. The most common reasons for initiating hormone replacement therapy were to help normalize irregular menstrual cycles and to assist in pubertal development (Table 1). Figure 10 illustrates height percentiles of

volunteers with galactosemia as a function of hormone replacement therapy use. Volunteers who reported using hormone replacement therapy had slightly higher median height percentiles compared to patients who did not undergo therapy, although this observation was not tested for statistical significance.

Additional puberty information collected from the surveys showed that 9 out of 27 volunteers with classic galactosemia reported having delayed thelarche, or reaching the second Tanner stage of breast development by age 14 years or older. Furthermore, 9 out of 29 volunteers reported having delayed menarche, with a first menstrual period occurring at 16 years or older.

Table 1. **Hormone replacement therapy (HRT) information from survey results**

Survey Question	Volunteers' Answers
Have you been on HRT?	
Yes	22
No	18
Omit	1
Age therapy was initiated?	
Min	10 years
Max	26 years
Avg.	16.4 years
Did you have your first period before HRT?	
Yes	14
No	4
Omit	4
Reason for initiation of therapy?	
Irregular periods	11
Birth control	0
Menopausal-like symptoms	2
Puberty help	10
Other	3 (primary ovarian insufficiency, abnormal FSH and LH, amenorrhea)

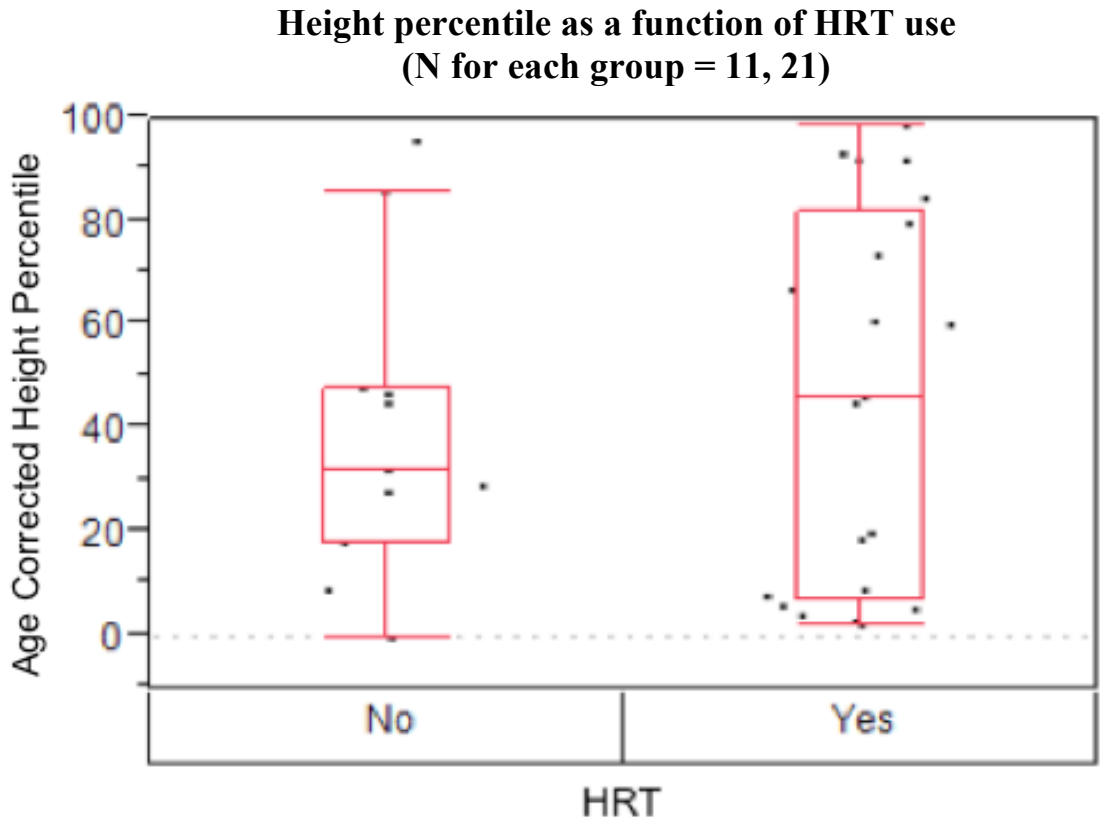

Figure 10. For each of the two data sets, box and whisker plots illustrate the median (center line in box), limits of the 25th and 75th percentiles (bottom and top of box), and 95th percentile confidence limits (bottom and top whiskers). Female patients who reported using hormone replacement therapy had slightly higher median height percentiles compared to patients who did not undergo therapy.

Discussion

Delayed postnatal growth

One of the reported long-term complications of classic galactosemia is delayed postnatal growth. The purpose of this investigation was to characterize the growth patterns of girls and women with classic galactosemia from childhood to adulthood**.** Additionally, the study aimed to evaluate whether relationships exist between patient growth, predicted residual GALT activity, ovarian function, and use of hormone replacement therapy.

Results from the cohort of volunteers showed that girls with galactosemia between the ages of 3 and 14 years had median heights below the 20th percentile, but that as they grew older their median heights reached above the 50th percentile (Figure 2). Additionally, affected girls and women between the ages of 3 and 24 years had median BMIs between the 20th and 40th percentiles (Figure 3). These findings were consistent with previous studies that had demonstrated decreased height-for-age and weight-for-age (Waggoner et al 1990) and decreased height velocity (Panis et al 2007) in girls with classic galactosemia.

Achieving normal outcome

Although delayed postnatal growth has been reported in girls with galactosemia, previous studies have also demonstrated that individuals with galactosemia can reach normal heights as they continue to age and grow. In this investigation's population of study volunteers, there were twenty control siblings and fifteen women with classic galactosemia over the age of 20 years. When height standard deviations and BMI percentiles were compared across the two groups, very similar median values were seen. Specifically, both control siblings and women with galactosemia over the age of 20 years had median heights between 0 and 1 standard deviation from the general population median (Figure 8). Additionally, both groups had median BMIs at nearly the 50th percentile (Figure 9). Again, these results were consistent with previous reports concerning classic galactosemic patients' abilities to reach normal heights and weights after experiencing delayed postnatal growth as children (Waggoner et al 1990; Panis et al 2007).

One could argue that "unaffected" siblings, who each have a 2/3 chance of being a carrier of a *GALT* allele associated with classic galactosemia, may not have completely normal growth outcomes themselves and consequently may not be ideal controls. In theory this could be a valid concern; however, because siblings' median height was between 0 and 1 standard deviation from

the general population median and their median BMI was at nearly the 50th percentile for the general population, it may be safe to predict that unaffected siblings of individuals with classic galactosemia do experience normal growth outcomes as adults. Of course, this result is also consistent with the idea that galactosemia is an autosomal recessive disorder. There have also been prior studies on carriers of galactosemia to investigate if they experience complications similar to individuals with the disorder. One study showed that serum AMH levels of carriers of galactosemia are normal (Knauff et al 2007). These findings were consistent with the observation that heterozygotes do not show any clinical evidence of ovarian insufficiency (Sanders et al 2009). Overall, evidence from prior studies supports that carriers of galactosemia are indeed unaffected and therefore may serve as useful and appropriate controls when studying outcomes of the disorder.

Growth outcome and AMH levels

It has been hypothesized that the growth delay observed in girls with galactosemia may be a consequence of the hormonal background that is associated with ovarian insufficiency (Waggoner et al 1990). In light of this concern, in this study we compared the growth outcomes of girls with AMH levels $<0.1\mu g/L$ and $\geq 0.1\mu g/L$. Whether a relationship existed between growth in girls and their AMH levels posed an intriguing question to address, because AMH can be used as an indicator of ovarian function in galactosemia (Sanders et al 2009). Furthermore, AMH is valuable when examining individuals with galactosemia, because the hormone's levels are relatively constant through a woman's menstrual cycle and also essentially unaffected by therapies such as oral contraceptives and gonadotropin treatments that are common among post-pubertal girls and women with classic galactosemia (Sanders et al 2009).

Twenty-one study volunteers with galactosemia who had provided their heights and weights also previously had their plasma AMH levels measured. Of the twenty-one volunteers, only five fit into the category of having AMH levels ≥0.1μg/L. A small percentage of volunteers having AMH levels ≥0.1μg/L is to be expected, because primary ovarian insufficiency, which is often accompanied by undetectable AMH, is seen in >80% of girls and women with classic galactosemia (Sanders et al 2009; Fridovich-Keil 2006; Waggoner et al 1990). Evidence of this is shown in a recent study of primary ovarian insufficiency in classic galactosemia; all twenty-four study volunteers had AMH levels below the detection limit of 0.1μg/L (Gubbels et al 2012).

Figures 6 and 7 indicate that AMH levels do not appear to influence height or BMI in girls and women with classic galactosemia, although the number of subjects for whom we had both growth data and AMH level was limited. In the future, it will be important to expand the volunteer population in this comparison to test whether the apparent lack of a relationship between AMH level and growth outcome is maintained. Currently, the Fridovich-Keil lab has determined AMH levels for over one hundred patient volunteers; however, only eighteen of these volunteers have also provided the lab with their growth information. To enhance the comparison of AMH levels to growth outcome, in the future it will be important to recruit volunteers with already determined AMH values into the growth study. Particularly, collecting growth information from the limited group of patients who have AMH levels ≥0.1μg/L should be emphasized.

Growth outcome and hormone replacement therapy

It has been hypothesized that suboptimal hormonal replacement therapy in girls with classic galactosemia might lead to their observed delayed growth (Panis et al 2007). Often, girls with galactosemia require estrogen or progesterone replacement therapy to achieve normal

secondary sexual characteristics and regular menstrual cycles (Sanders et al 2009; Rubio-Gozalbo et al 2006). As shown in Table 1, of the forty-one volunteers who submitted puberty surveys, twenty-two reported using hormone replacement therapy. The average age at which HRT therapy was initiated was 16.4 years. The youngest and oldest reported ages of therapy initiation were 10 years and 22 years, respectively. The most common reasons for our volunteers' initiating hormone replacement therapy were to help normalize irregular menstrual cycles and to assist in pubertal development. Although hormone replacement therapy has been successful in assisting with these issues, this therapy could also potentially have adverse effects on patient growth if not applied carefully. For example, the estrogen exposure from certain hormone replacement therapies could lead to inadvertently impaired growth due to premature growth plate senescence (Weise et al 2004). Additionally, inadequate hormone replacement therapy for primary ovarian insufficiency could result in low bone mineral density (Batey et al 2012).

Taking into consideration the potential complications of hormone replacement therapy, data collected in this investigation were used to determine whether a relationship exists between growth outcome and hormone replacement therapy use. Heights of 21 volunteers who had reported using hormone replacement therapy were compared to the heights of 11 volunteers who reported they had not used any hormone replacement therapy. Interestingly, the median height percentile of the group of individuals that had reported using hormone replacement therapy was slightly higher, not lower than the median height percentile of the group that had not used therapy (Figure 10). However, the number of individuals included in this comparison was limited, and no statistically meaningful relationship can be inferred. Nonetheless, it is reassuring to observe that hormone replacement therapies did not seem to have an adverse effect on patient

growth, especially since the use of hormone replacement therapy is very common among galactosemic girls and women. Results of future investigations of the relationship between hormone replacement therapy use and growth outcome would be more informative if the number of volunteers in each category were increased substantially. Additionally, in light of Batey and colleagues' hypothesis, it would be interesting to determine if a relationship exists between hormone replacement therapy use and bone mineral density.

Growth outcome and predicted residual activity

Another question that has been addressed in previous studies is whether or not *GALT* genotype (Kaufman et al 1994; Tyfield 2000) acts as a modifier of patient outcome. Kaufman et al found that cognitive functioning, presence of neurologic symptoms, and the timing of ovarian insufficiency could not be predicted or explained based solely upon patient genotype. Somewhat similarly, Tyfield et al concluded that *GALT* variants play only a partial role in defining clinical phenotype.

In this study, rather than *GALT* genotype, predicted residual GALT activity based on a yeast expression system was studied and related to patient growth outcome. An identical approach was taken in a recent study, except that predicted residual GALT activity was related to scholastic outcomes; of note, results showed that predicted residual GALT activity did in fact appear to modify scholastic outcome (Ryan et al 2013). In the scholastic study, individuals with predicted GALT activities ≥0.4% of wild-type function appeared to have better outcomes. A similar relationship between predicted residual GALT activity and ovarian outcome has also been observed (Spencer et al, submitted).

Figures 4 and 5 indicate that predicted residual GALT activity does not appear to influence height or BMI in girls and women with classic galactosemia, although the number of

subjects for whom we had both growth data and predicted residual GALT activity values was limited. Individuals with predicted residual GALT activity values ≥0.4% of wild-type function were especially rare (N=6) in this investigation. In the future, to expand upon the investigation of predicted residual GALT activity's potential impact on patient growth, it will be important to collect growth information from more individuals with calculated predicted GALT activity values, especially those volunteers with GALT activity values ≥0.4% of wild-type function.

Limitations

As often is the case in investigations of rare diseases, the pool of volunteers was limited in this study. To maximize the amount of growth information collected, growth charts were collected from some volunteers. Although this information was useful, in the AMH, residual GALT activity, and hormone replacement therapy analyses where the number of volunteers were further limited, it was important that each volunteer only had one data point per box and whisker plot. Although this was to ensure that a single volunteers' information would not be influencing the outcome of the results, it further reduced the N value in the analyses. The number of unaffected sibling controls was also limited in this study. Although there were thirty-five unaffected siblings who volunteered to participate in the study, only fifteen volunteers were under the age of 20 years. As a result, it was difficult to make comparisons between young girls and teenagers with galactosemia and the control siblings.

Future Direction

This study served as a pilot investigation of the effects of predicted residual GALT activity, plasma AMH level, and hormone replacement therapy use on growth outcome of girls and women with classic galactosemia. In the future, I would like to continue this investigation through expanding the number of both the unaffected siblings and girls and women with classic

galactosemia enrolled in the study. Ideally, we would recruit enough volunteers so that we could compare heights and BMIs of individuals with galactosemia to their unaffected siblings across all ages. Additionally, with more volunteers, we would potentially be able to yield statistically significant results from our investigation. Furthermore, in addition to studying growth outcome in girls and women with galactosemia, we would like to conduct similar investigations in boys and men with galactosemia.

It would also be useful to determine and investigate other growth markers such as IGF-I and IGFBP-3 that might influence growth outcome. Through these studies, we hope to obtain a more thorough understanding of the physical growth patterns and biochemical factors that may contribute to growth in children and adults with galactosemia.

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